MICROBIOTIX Inc.

MBX-400 – Treatment and prevention of human cytomegalovirus (HCMV)-related disease

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Microbiotix, Inc.
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Microbiotix, Inc.

- **Located in Worcester, MA**
  - 10,700 sq. ft. of office space, and fully equipped BSL2 microbiology and medicinal chemistry laboratories
  - 20 employees: Microbiologists and medicinal chemists
  - Anti-viral and anti-bacterial small molecule drug discovery & development
  - Large number of expert academic collaborators
  - Experienced pharmaceutical, biotechnology and regulatory R&D management team

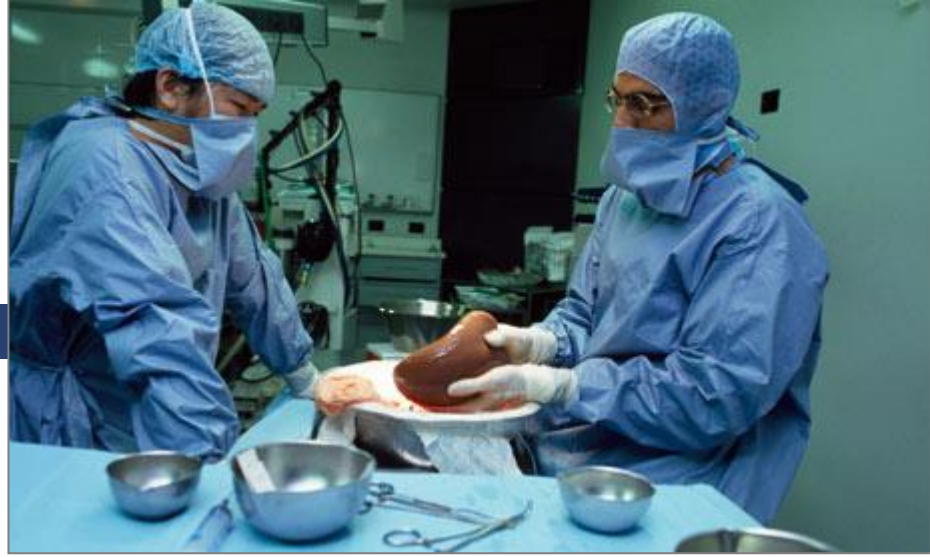
Microbiotix Scientific Objectives

- **Pre-clinical candidate identification:** Research proof-of-concept (POC) studies conducted by Microbiotix (Worcester, MA) and collaborators
- **Clinical candidate identification:** GLP IND (Investigational New Drug) enabling studies outsourced to CROs with Microbiotix oversight
- **Regulatory:** IND submission (Microbiotix)
- **Clinical proof-of-concept** (POC): Human clinical phase I safety and phase II efficacy studies outsourced (CROs) with Microbiotix oversight

Microbiotix: Development Portfolio

- **Two pre-clinical anti-bacterial programs:**
  - MBX-900: Novel broad spectrum antibiotic for the treatment of bacterial resistance
  - MBX-3323: Prevention of bacterial virulence (T3SS) mediated immunosuppression (**NIAID SBIR supported**)
- **Two anti-viral programs in human clinical trials:**
  - MBX-700: HCV-related liver disease
  - MBX-400: HCMV-related disease (**NIAID SBIR supported**)
  - Both MBX-700 and MBX-400 have successfully completed Phase Ia human safety studies
  - Phase Ib studies planned for 4Q14 for both compounds

Human Cytomegalovirus (HCMV)-Related Disease



Unmet Medical Needs:

- Primary HCMV at-risk populations:
  - Immunocompromised (transplant, AIDS, cancer)
  - Congenital (leading cause of deafness in children)
- Need alternative to the standard ganciclovir:
  - Less toxic (e.g. bone marrow, etc.)
  - Better long term safety and overall tolerability
  - Active against ganciclovir-resistant HCMV

HCMV Market

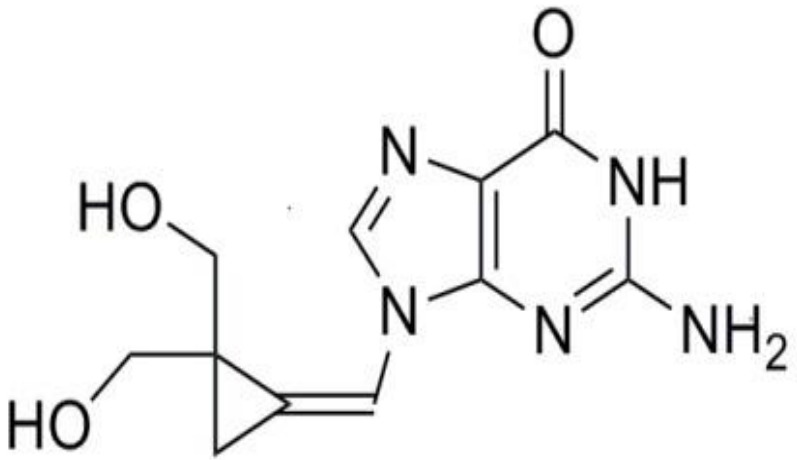
- **Growing market:** Estimated world wide sales to reach >**$1 billion** by 2019*
- 4 currently marketed products:
  - Ganciclovir (Cytovene®): Roche + generics
  - **Valganciclovir (Valcyte®): Roche 90% of market**
  - Foscarnet (Foscavir®): AZ (renal toxicity limits utility)
  - Cidofovir (Vistide®): Gilead (renal toxicity limits utility)

*source: GlobalData, January 2013

HCMV Inhibitors In Development

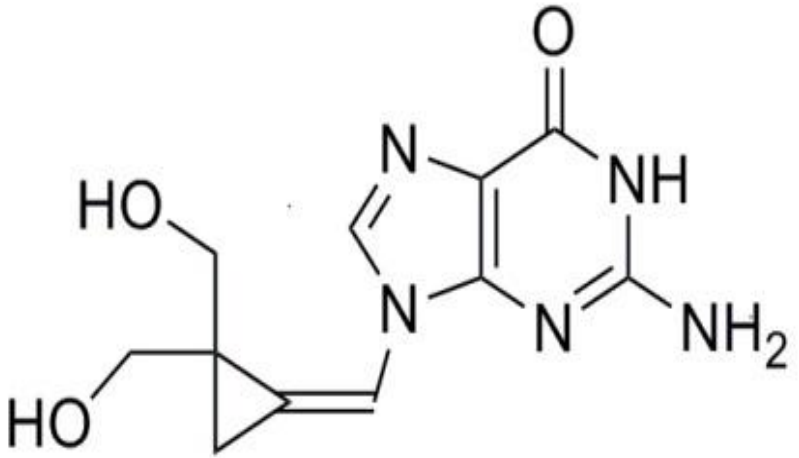
- AIC246 (AiCuris – now Merck): Non-nucleoside terminase inhibitor in human Phase III studies
- CMX001 (Chimerix): cidofovir lipid conjugate in human Phase III studies
- RG7667 (Roche): MAb in human Phase II studies
- MBX-400 (Microbiotix): Nucleoside in human Phase I safety studies.

MBX-400: Prevention/Treatment HCMV Disease



- Nucleoside (guanosine)
- Methylenecyclopropane analog
- Triphospate is active form
- Molecular weight: 263.25

MBX-400: SBIR Goals



- SBIR Phase I award to carry out medicinal chemistry and virology to **identify pre-clinical anti-HCMV candidate**
- SBIR Phase II award to carry out IND enabling GLP pre-clinical studies to **identify clinical anti-HCMV candidate**

MBX-400: Pre-Clinical Studies carried out in NIAID SBIR Phase I (R43 AI054135)

Medicinal Chemistry

- Structure activity relationship (SAR) determined against beta (CMV/HHV6) and gamma (HHV8) herpes viruses
- Optimize research synthetic route for future human clinical scale-up needs

Virology

- Anti-HCMV efficacy *in vitro* and *in vivo*
- Resistance
- Mechanism of action

MBX-400 (ZSM-I-62): Methylenecyclopropane analogs are active against beta (CMV/HHV6) and gamma (HHV8) herpesviruses

Compound	EC_{50} (µM) for:									CC_{50}
	HSV-1	HSV-2	VZV	HCMV	MCMV	HHV-6A	HHV-6B	EBV	HHV-8	
ZSM-I-32	>404	>404	>404	12	10	18 ± 23	4.6 ± 3.5	166	57 ± 34	>404
ZSM-I-58	>448	>448	>448	14	54	18 ± 16	23 ± 1.6	>45	197 ± 38	>448
ZSM-I-62	>380	>380	>380	1.2 ± 0.8	0.3	7.8 ± 1.8	0.7 ± 0.7	45	6.5 ± 0.4	>380
ZSM-I-64	>380	>380	>380	123	42	116 ± 22	43 ± 50	119	>190	>380
ZSM-I-158	>361	>361	3	2.7	2.0	28 ± 4.1	2.3 ± 1.2	161	16 ± 2.8	>361
ZSM-I-89	>420	>420	>420	>420	NT[e]	NT	NT	4.4	5.5 ± 2.0	>420
ZSM-I-287	>381	>381	39	3.3	NT	NT	NT	<0.3	>165	>381

MBX-400: More Active *In Vitro* Against HCMV than the Standard Ganciclovir

Relative potency

CMV Strain	EC_{50} (μM)	
	MBX-400	Ganciclovir
AD169	**1.3 ± 0.7**	5.5 ± 3.5
Davis	**1.0 ± 0.9**	5.9 ± 1.9
Toledo	**1.3 ± 1.2**	8.2 ± 6.3
Coffman	**1.9 ± 0.3**	15.3 ± 11.8

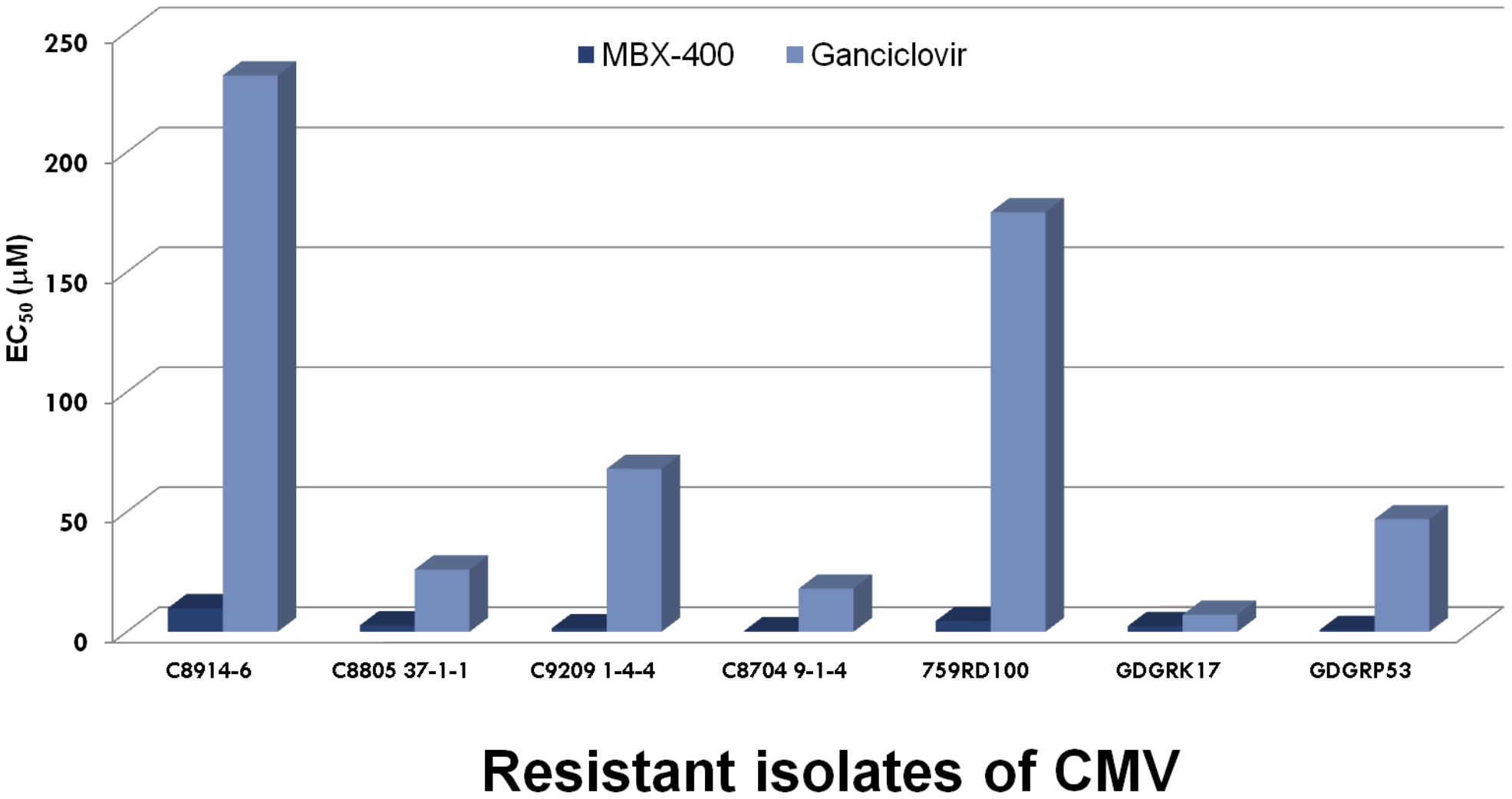
>4 fold more potent

Activity spectrum

Virus	MBX-400 EC_{50} (μM)
CMV	1.2 ± 0.8
HHV-6A	7.8 ± 1.8
HHV-6B	0.7 ± 0.7
HHV-8	6.5 ± 0.4

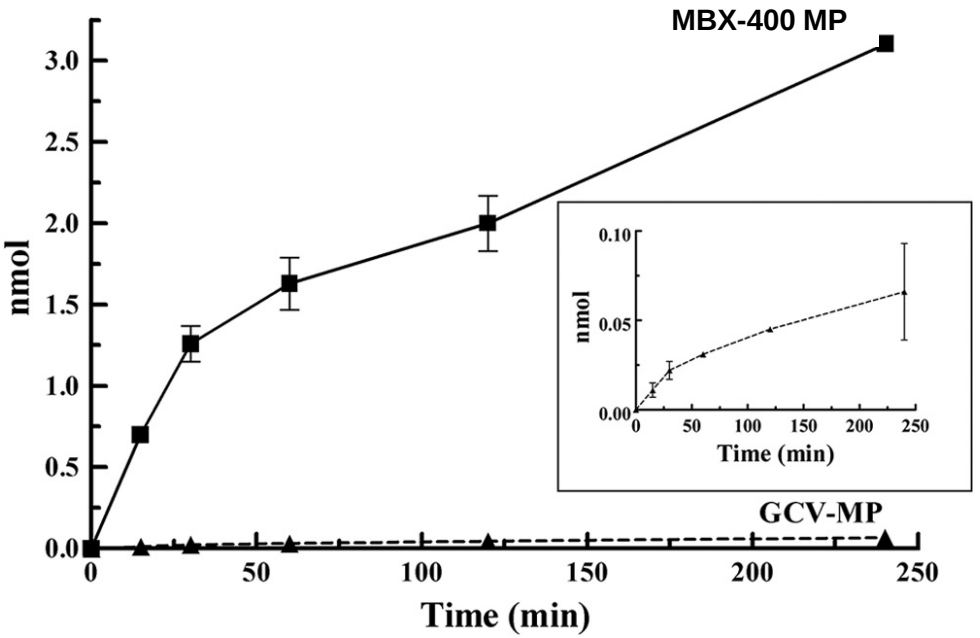
Active against HHV6/8

MBX-400: Retains Potency Against Ganciclovir Resistant Clinical Isolates

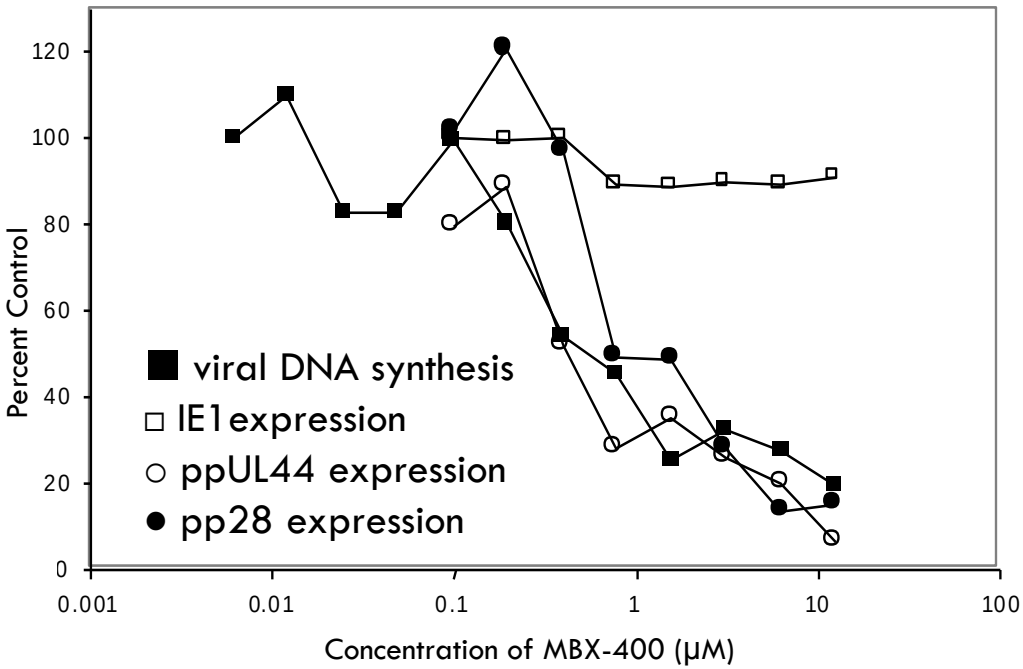


MBX-400: Mechanism of Action

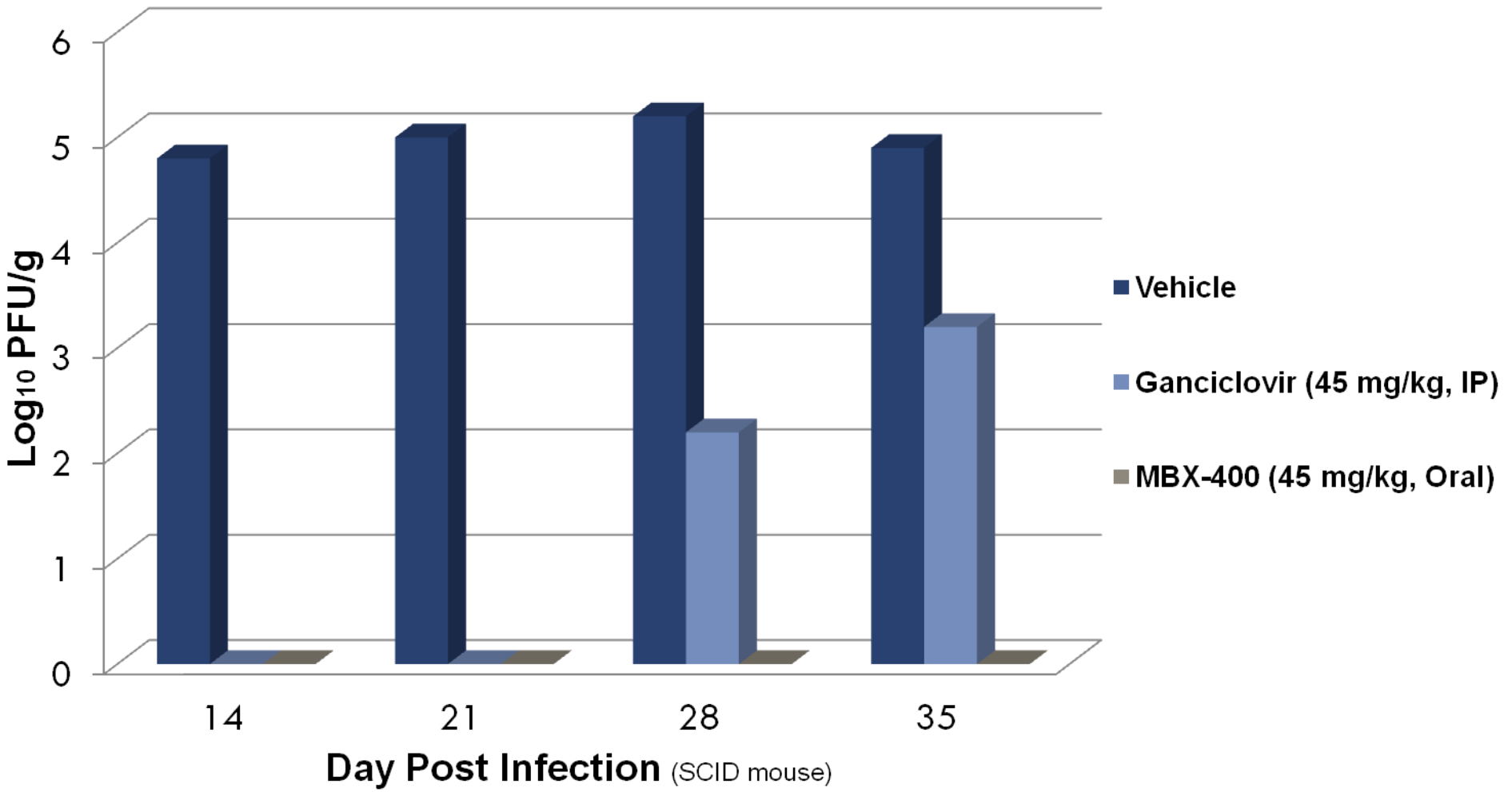
- MBX-400 is converted to its monophosphate [MBX-400 MP]
- Accumulation of MBX-400 MP approximately 45-fold greater than GCV-MP



MBX-400 Inhibits HCMV DNA Synthesis



MBX-400: More Active in HCMV Animal Models Than the Standard Ganciclovir



MBX-400: Pre-clinical Studies performed in SBIR Phase II (R44 AI054135)

- **Safety Pharmacology**
  - Cardiovascular
  - Respiratory
  - CNS
- **Pharmacokinetics (PK)**
  - PK studies rat and dog
  - Plasma protein binding
  - Cytochrome P450 induction/inhibition
- **Toxicology**
  - Single dose and 14-day repeat dose studies in rats and dogs
  - Genetic toxicology (Ames, CHO, micronucleus, comet)

MBX-400: Low Risk for Cardiotoxicity determined in hERG Assay

- hERG channel: *In vitro* screen for cardiotoxicity
- FASTPatch™ Profiling Screen
  - K+ channel current; automated patch clamp
  - HEK293 cells transfected with hERG DNA

Test Article	Concentration (µM)	Mean hERG Inhibition (%)
E-4031 (positive control)	0.5	99.2
MBX-400	10	1.2
MBX-400	50	4.9

MBX-400: No Undesired Effects in Cardiovascular Safety Pharmacology Studies

- Dog Cardiovascular Safety Pharmacology Study Design:
  - MBX-400 dosed at 1-50mg/kg (see schedule)
  - Evaluated at 1.25 hours post-dose: PR interval, QRS duration, **QT interval**, blood pressure, mean arterial pressure, pulse pressure and heart rate
  - ***Conclusion: MBX-400 had no effects on any cardiovascular parameters***

Animal	Oral Dose Level Designation on Specified Dosing Days (mg/kg)					
	Day 1	Day 8	Day 23	Day 30	Day 37	Day 44
1	1	0	50	10	-	-
2a	10	50	-	-	-	-
2b	-	-	0	1	10	50
3	50	1	10	0	-	-
4	0	10	1	50	-	-

MBX-400: No Undesired Effects in Respiratory or CNS Safety Pharmacology Studies

- Respiratory Safety Pharmacology (Dog)
  - Method: Evaluated whole-body plethysmography for two 15-minute intervals pre-dose and four 15-minute intervals post-dose: respiratory rate, tidal volume and minute volume
  - ***MBX-400 had no effects on respiratory parameters***
- CNS Safety Pharmacology (Rat)
  - Method: Evaluated modified Irwin observational battery pre-dose and 0.5, 2, 4, 8 and 24 hours post-dose: home cage observations, hand held observations, open field observations and elicited responses
  - ***MBX-400 had no effects on neurologic responses***

Respiratory Safety Pharmacology Study Design

Group	IV Dose (mg/kg)	Number of Male Sprague-Dawley Rats	[MBX-400] (mg/mL)
Vehicle	0	4	0
MBX-400	30	4	3
	60	4	6
	90	4	9

CNS Safety Pharmacology Study Design

Group	Oral Dose (mg/kg)	Number of Sprague-Dawley Rats		[MBX-400] (mg/mL)
		M	F	
Vehicle	0	6	6	0
MBX-400	10	6	6	1
	50	6	6	5
	100	6	6	10

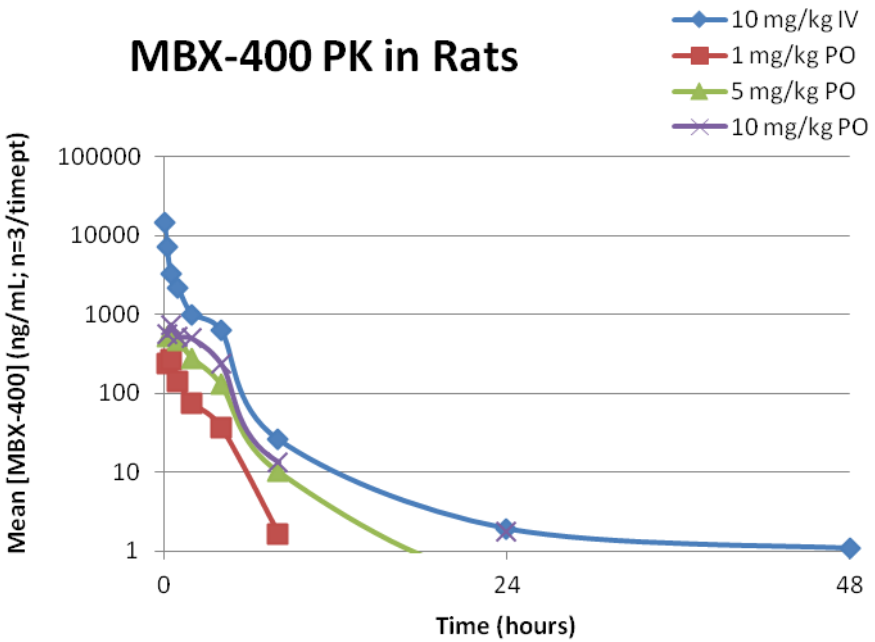
MBX-400: Orally Bioavailable in Rats and Dogs

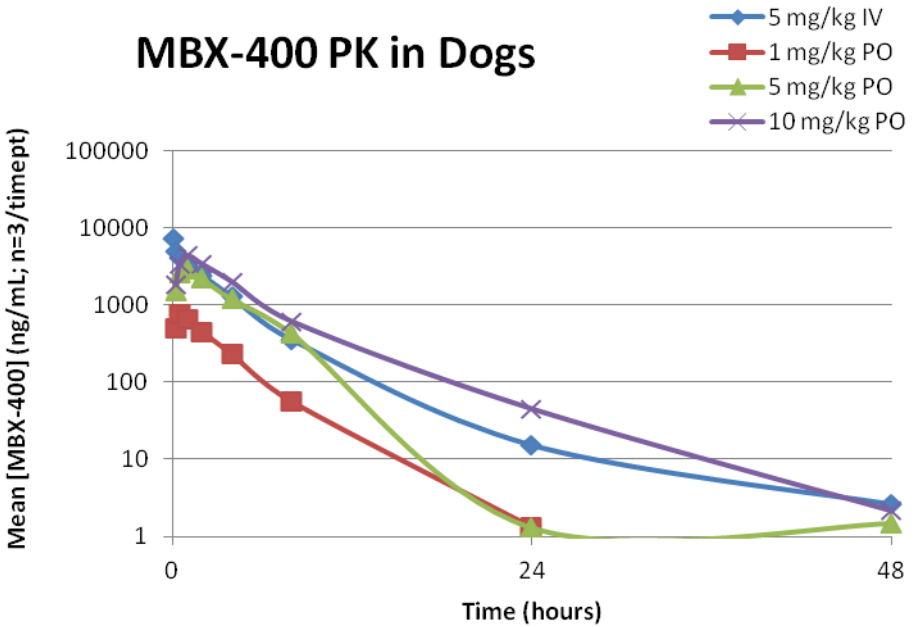
5 mg/kg PO

C_{max} = 590 ng/mL
T_{max} = 0.5 hr
$AUC_{0-\infty}$ = 1638 ng*hr/mL
$t_{1/2}$ = 1.23 hr

5 mg/kg PO

C_{max} = 2970 ng/mL
T_{max} = 0.833 hr
$AUC_{0-\infty}$ = 14800 ng*hr/mL
$t_{1/2}$ = 8.48 hr





- Oral bioavailability was higher in dogs than in rats at doses of 1, 5 and 10 mg/kg (84.8, 91.1 and 70.8 and 46.0, 30.5 and 22.7, respectively)

MBX-400: Not Highly Protein Bound and Excreted Primarily In Urine Unchanged

- **Plasma Protein Binding:**
  - Method: Dialysis for 2 hours in rat, dog and human plasma at 5 and 30 µM MBX-400; solid phase extraction; LC-MS/MS analysis
  - Rat: 27.75 and 28.78%; Dog: 29.31 and 25.98%; Human:19.59 and 18.77%
  - ***MBX-400 had relatively low protein binding***
- **Cytochrome P450 Induction and Inhibition:**
  - Induction was evaluated at 20 µM for CYP 1A2, 2B6, 2C9 and 3A4
  - Inhibition was evaluated up 50 µM for CYP 1A2, 2B6, 2C8, 2C9, 2C19, 2D6 and 3A4
  - ***MBX-400 did not cause induction or inhibition, except it weakly induced 2C9***
- **Metabolism:**
  - *In vitro* metabolism: No metabolites were induced when incubated in human hepatocytes or microsomes
  - *In vivo* metabolism: MBX-400 was excreted primarily unchanged in urine (>99% parent present)
  - ***MBX-400 is not likely to be subject to first pass metabolism***

MBX-400: Low Potential for Genotoxic Effects

- **Ames**
  - Tested at concentrations up to 5,000 µg/plate
  - **No induction of reverse mutations**
- **Rat Micronucleus**
  - Doses of 500, 800 or 2,000 mg/kg x 1 dose
  - **Not cytotoxic to bone marrow**
  - **No induction of micronucleated polychromatic erythrocytes**
- **Chromosomal Aberration**
  - Structural chromosomal aberrations were observed (without metabolic activation at 240 µg/mL; with at 240, 343 and 700 µg/mL)
  - **No increase in cells with polyploidy or endoreduplication was observed**
- **Rat Comet**
  - Evaluated liver and kidney
  - Doses 500, 1,000, 2,000 mg/kg/day x 2 daily doses
  - **No evidence of DNA damage in liver and kidney cells**

MBX-400: Repeat Dose Toxicity Studies Established Safe Starting Dose in Human Safety Studies

- Rat: NOAEL 100 mg/kg in 14d oral toxicity study
- Beagle dogs: 14 day repeat oral
  - NOAEL: 10 mg/kg/day non-fasting
  - Target organs: kidney, GI tract, bone marrow (consistent with chemical class)
- **Maximum safe starting dose in humans**
  - Human equivalent dose (HED) = animal dose in mg/kg*(animal wt in kg/human weight in kg)$^{0.33}$; or 5.4 mg/kg/day
  - Safety factor = 10 = 0.54 mg/kg/day
  - Adjust for 70 kg subject = **37.8 mg/day**

MBX-400: Chemistry Manufacturing and Controls (CMC)

- **SBIR support:**
  - Improved synthetic scheme suitable for scale-up (e.g. chromatography step replaced by crystallography)
  - Synthetic scale improved to produce >100g non-GMP material
- **NIAID RAID** (Rapid Access to Intervention Development) **support:**
  - Synthetic scheme transferred and >1.0 Kilo GMP material produced at Ash Stevens, Detroit MI.

MBX-400: Summary of SBIR Phase I/II Studies Successfully Submitted in IND

- **Virology**
  - Efficacy
  - Resistance
  - Mechanism
- **Safety Pharmacology**
  - hERG
  - Cardiovascular
  - Respiratory
  - CNS
- **Pharmacokinetics** (PK)/Toxicokinetics/ (TK)/ *In vitro* ADME
  - Rat and dog PK studies, including bioavailability
  - Plasma protein binding
  - Cytochrome P450 induction/inhibition
- **Toxicology**
  - Genetic toxicology (Ames, CHO, micronucleus, comet)
  - Single dose and 14-day repeat dose studies in rats and dogs

MBX-400: Human Phase 1A Safety Study Design

- Six cohorts of 8 subjects (6 active, 2 placebo)
- Escalating doses (35, 100, 350, 700, 1000, 1350 mg; capsules filled at local pharmacy)
- Key inclusion criteria:
  - Male/female 18-65
  - BMI 18-32 kg/m^2
- Key exclusion criteria:
  - Significant medical problems
  - Clinically significant EKG, laboratory results, vital signs

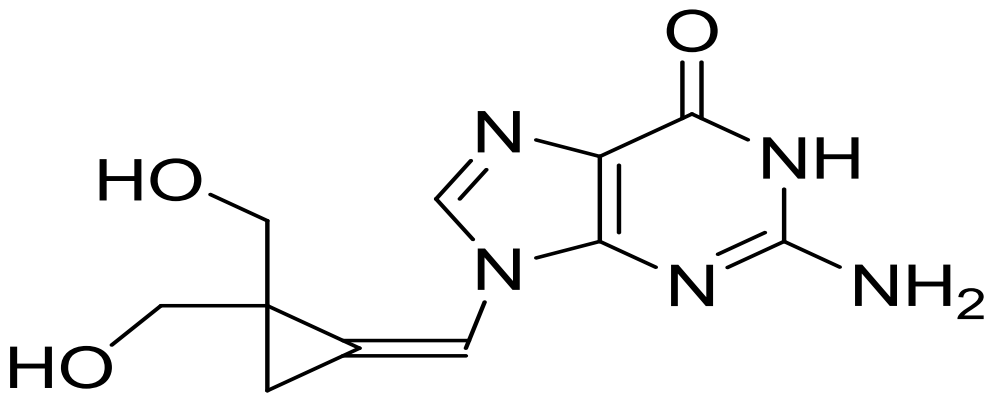
MBX-400: Phase Ia Study Endpoints

- Safety
  - Serious or life-threatening adverse events from treatment to the end of the study
  - Evaluation of all adverse events, laboratory data, electrocardiograms, vital signs, weight and physical exams
- Establish the pharmacokinetic profile of MBX-400 in healthy adult subjects

MBX-400: Safe and Well Tolerated in a Human Phase Ia Clinical Safety Study

- **Single oral doses ranging from 35 to 1350 mg were extremely safe and well-tolerated**
- **No safety issues were identified**
- **All 48 subjects completed the study**
- **Good oral bioavailability**

MBX-400: Development Status



- Regulatory: Orphan Drug designation granted
- Intellectual Property: Issued US and international patents and patents pending
- Human Phase Ia Safety Study: Safe and well tolerated
- Human Phase Ib Multiple Dose Safety Study: 4Q14

MBX-400: Acknowledgements

Collaborators:

- Earl R. Kern, Ph.D, Mark N. Prichard, Ph.D. and Richard J. Whitley, MD, University of Alabama, Birmingham
- Jiri Zemlicka, Ph.D., Wayne State University
- Sunwen Chou, MD, Oregon Health Services University

National Institute of Health:

- National Institute of Allergy and Infectious Diseases (NIAID): SBIR phase I and II award (R43/44 AI054135)
- Rapid Access to Intervention Development (RAID) program: GMP clinical material
- NIAID VTEU clinical program: Human Phase Ib safety study

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health

MICROBIOTIX Inc.

Discovering and Developing Treatments for Serious Infectious Diseases

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